## 2833 δ-Hemolysin in *Staphylococcus lugdunensis* of Acute Oral Infection.

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To investigate *Staphylococcus lugdunensis* (*S. lugdunensis*) in acute oral infection, staphylococci were isolated and characterized.

*S. lugdunensis* was isolated only from patients with acute oral infection. DNA isolated from *S. lugdunensis* showed a positive reaction with the δ-hemolysin gene probe of *S. aureus* in a dot blot analysis, but a 7.3 kb *Hin*dIII fragment was observed in the DNA of *S. lugdunensis* that gave synergistic hemolysis in a Southern blot analysis. The molecular size of partially purified δ-hemolysin in *S. lugdunensis* was about 50 kd. The cloned fragments from the chromosomal DNA of *S. lugdunensis* showed the partial homology with the insulin receptor-related and dopamine receptor of humans.

These results suggest that *S. lugdunensis* might be an important pathogen in acute oral infection and show some homology with eukaryotes. This paper was supported by KOSEF 961-0702-021-2.

## 2834 *In vitro* study of the effect of metronidazole and spiramycin on *Porphyromonas gingivalis* in the presence of titanium.

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In view of the conflicting results on the antibacterial effect of titanium reported in the literature, this study analysed, *in vitro*, how *P. gingivalis*, which is involved in implant failures, reacts in the presence of spiramycin and metronidazole alone and in combination (ratio 2/1) and of titanium. *P. gingivalis* (ATCC 33277 or W83) cultures ($5.10^7$ bacteria/mL) were distributed into 6 tubes of Todd-Hewitt broth enriched with hemin and vitamin K1. Tubes 1 and 2 were used to establish a two-point standard curve: 0 and 100% viability. Either 0.07µg/mL of metronidazole or 0.14µg/mL of spiramycin, or both, were added to tube 3. A 14 mm, 0.5 mm thick titanium disk was placed in tube 4. Tube 5 was used to measure the effect of the antibiotics and the titanium in combination, while tube 6 contained a nickel-chrome disk (control). After an 18 hour incubation, all the bacterial suspensions were adjusted to $2.10^8$ bacteria/mL. Viability was determined, versus the standard curve, in 96 well microplates using the Live/dead Baclight Bacteria Viability Kit™. The results (10 measurements/tube) of each manipulation were compared to each other (Student and Fisher tests). The results of all 48 experiments (60 measurements each) were compiled in a database and compared to each other using the $\chi_2$ $p<0.05$ test. Frequencies rather than absolute values were used given the variations in the growth capacity of anaerobic bacteria. A comparison of the wells containing no antibiotics with those containing antibiotics, whether in the presence of titanium or not, showed that the antibiotics had an effect on bacterial growth $\chi_2=0.08$, ddl=1. A comparison of the wells containing no antibiotics with those containing only titanium disks showed that the titanium enhanced bacterial growth. However, titanium was shown to potentialize the effect of the antibiotics $\chi_2=9.14$, ddl=1. With metronidazole alone, the effect was much clearer $\chi_2=11.05$, ddl=1 than with the antibiotic mixture $\chi_2=6.74$, ddl=1. The titanium did not potentialize the effect of the spiramycin $\chi_2=0.07$, ddl=1. The control was similar to the titanium $\chi_2=0$, ddl=1. These results can explain the conflicting data described in the literature. When titanium is used on its own, it enhances bacterial growth. When it is used in the presence of metronidazole, it potentializes the effect of the antibiotic. When the antibiotic has no regular effect (as with spiramycin), there is no potentialization and the results are the same as if there were no antibiotic present. This study was supported by SPECIA/Groupe RHONE-POULENC RORER.

## 2835 Postantifungal effect elicited by five antifungal agents in oral *Candida albicans*.

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Limited exposure to antibiotics and subsequent removal of the drug leads to profound but usually transient changes in the exposed organisms. This phenomenon is known as the postantibiotic effect (PAE). As there are only a handful of studies on the postantifungal effect (PAFE) in *Candida albicans* isolates we evaluated the PAFE of five antifungal drugs namely nystatin (NY), amphotericin B (AB), 5-fluorocytosine (5FC), ketoconazole (KZ) and fluconazole (FZ) against ten oral *Candida albicans* isolates. After determination of the minimum inhibitory concentration (MIC) of the drugs using the broth dilution technique, cell suspensions of $10^6$-$10^7$ yeasts/ml were exposed to sub-cidal concentrations of antifungal drugs at ×4-8 MIC for 1hr at 37°C, in RPMI broth. This was followed by removal of the drug by two cycles of centrifugation, complete decanting of the supernatant and resuspension of pellets in sterile PBS. Aliquots of 100µl from each suspension was added to microtitre wells containing 150µl of RPMI broth. Thereafter the PAFE was assessed with the help of a Spectramax machine which utilizes the principle of periodic turbidometric assessment of growth rates automatically at 37°C over 18 hr. The data thus collected are automatically processed in a graphic format as a computer print out. The PAFE was determined as the difference in time required for growth of the drug-free control and the drug-exposed test cultures to increase to a defined absorbance level following removal of the antifungal drug. The PAFE of NY, AB, 5-FC, KZ and FZ were 2.89 (± 0.27), 2.83 (± 0.23), 3.18 (± 0.31), 0.65 (± 0.11) and 0.16 (± 0.06) hr, respectively. Exposure to NY, AB and 5FC showed a significant PAFE while a marginal PAFE was observed for KZ and little or none for FZ. These findings may have important relevance for the antifungal dosage regimens against both superficial and systemic candidal infections, since antifungal agents inducing long PAFE's can be administered with longer dosing intervals.

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## 2836 Anti-Candidal Activity of Human Gingival Keratinocyte Cytosol.

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The mucosal epithelia is constantly exposed to microorganisms. Antimicrobial protection is conferred at least in part by the secretions bathing these epithelial surfaces. While host mucosal tissues express antimicrobial peptides, it is not known if the cytosolic contents of human gingival keratinocytes (HGKs) actually contain antimicrobial activity. In this study the anti-fungal activity of HGK cytosol was compared to a non-cytosol containing control and the cytosol of white blood cells (WBC). HGKs cultured from healthy gingival tissue were collected by trypsinization and washed in Hanks' Balanced Salt Solution (HBSS) before being resuspended in 500 µl of HBSS. The cells were then disrupted by sonication on ice. Insoluble cellular material and debris was pelleted by centrifugation (5 min at 12,000 x g). After lysis of red blood cells with isotonic ammonium chloride, WBC were pelleted, washed with HBSS and cytosol was isolated as for HGK. The supernatant (cytosol) of each cell type was decanted and tested for anti-fungal activity in a microtitre plate assay with *Candida albicans*. Doubling dilutions of cytosol were plated in triplicate followed by addition of 25µl of Sabouraud-dextrose broth containing $10^4$/ml *C. albicans* (final well volume 100µl). Control wells contained 75µl of HBSS with the 25µl of Sabouraud-dextrose broth/*C. albicans*. After overnight aerobic incubation at 37°C, the cells in each well were suspended uniformly by repeated pipetting and counted. As expected, all dilutions of the WBC cytosol inhibited the growth of *C. albicans*. At similar protein concentrations, HGK cell cytosol showed about 1/1000 of the anti-fungal activity in WBC causing a 5-fold decrease in the growth of *C. albicans* compared to controls without cytosol (Students' t test, $p < 0.0015$). These data show that like WBC, HGK cytosol may contain antifungal activity. This study was supported by NIH grant 1R01-DE11831

## 2837 Efficacy of two prophylactic procedures assessed by Dentocult SM-method.

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The aim of this study was to assess efficacy of two different preventive procedures in reduction of oral microflora. The quantity of Streptococcus mutans (MS), Lactobacillus (LB), and buffer capacities of saliva (DB) were measured by Dentocult tests (Vivadent, Schan, Liechtenstein). The study group comprised 36 children divided in two equal groups according to age (4-5 and 10-12 years). The first group was treated with topical fluoride (Aminflorid), and the second one with prophylactic paste (Proxyt, Vivadent). The number of colony forming units (CFU) was assessed before the procedure, and 30 min, 7 days, 30 days, and 60 days after application of agent. Findings in the first group have shown reduction of LB number to $10^3$ CFU/ml in the second reading (after 30 min), and $10^4$ CFU/ml in third reading (after 7 days) comparing to the starting position of $10^5$ CFU/ml. The number of SM decreased from $10^6$ CFU/ml before treatment to $10^4$-$10^5$ CFU/ml after the preventive procedure. Number of bacteria in the group treated with prophylactic paste stayed at the same level after 30 days ($10^3$-$10^4$ CFU/ml for LB, and $10^4$-$10^5$ CFU/ml for SM). One month after application of topical fluoride mutans streptococci reached the baseline level, while two months after use of prophylactic paste the level of MS and LB was only 20% higher than immediately after preventive procedure. The results show that the prophylactic paste Proxyt has superior effect on reduction of mutans streptococci in saliva comparing to the topical application of fluoride solution.

## 2838 Photoinactivation of *Prevotella nigrescens*.

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The aim of the study was to investigate the effect of continuous wave (CW) light sources on the viability of *Prevotella nigrescens* (Pn) cells following photosensitisation with malachite green isothiocyanate (MG). Previous studies by the authors have shown that Pn are killed with pulsed laser light from an Nd:YAG-pumped dye laser, following photosensitisation with MG, at pulsed energy 11 mJ. At 0.5 min exposure to laser light, % viability of Pn suspended in MG was 21.7±0.26. % viability in the absence of MG was 79.4±0.28. Pn cells in PBS and MG (at final concentrations of MG from 50 to 500 $\mu gml^{-1}$) were irradiated with light from the following; an Argon ion-pumped dye laser, a xenon lamp and an LED array. Light was emitted at λ630/640 nm. Samples were irradiated for 0.5, 1.0 and 5.0 min. Cell viability was measured by performing colony counts. Experimental plates were compared to controls. Results show that after exposure to light from the Argon ion laser, cell viability is 3.03±0.13% in the *absence* of MG at 0.5 min. After sensitisation with MG, viability is 36.2±0.13%. Similarly, after exposure to the xenon lamp % viability, without MG, was 0.0, with MG % viability was 29±0.139. After exposure to the LED for 0.5 min, cell viability was 0.27±0.33% without MG. With MG, viability was 7.34±0.04%. In general, cell viability was reduced as radiation dose increased. MG had a toxic effect on cells at concentrations >300 $\mu gml^{-1}$. MGITC does not appear to be activated by CW light in the same way as by pulsed laser light. However, *Prevotella nigrescens* can be killed using CW laser light.

## 2839 Effect of ThreeAntimicrobial Mouthwashes on Bacterial Adhesion to Restoratives

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The aim of this *in vitro* study was to investigate the effect of various antimicrobial mouthwashes on the bacterial adhesion to different dental restorative materials. Dyract (Dentsply, DeTrey), a polyacid-modified resin composite, and Vitremer (3M), a resin-modified glass-ionomer were evaluated. The samples of the materials were prepared as discs (5 mm x 2 mm) and stored in sterilized saliva for 5 min. Then, they were treated with Oral B (cetylpyridiniumchloride , Oral B), Colgate Plax (Triclosan, Colgate-Palmolive) or Chlorhexamed Fluid (Chlorhexidindigluconat, Blend-a-med Forschung,) for 1 min. As the positive control, a bacterial suspension of *Streptococcus mutans CCUG 11877* was incubated in tryptic soy broth for 1, 3, 24 and 48 hr at 37°C in 5 % $CO_2$ containing atmosphere. The samples were immersed into the bacterial suspension directly or after mouthwash treatment, respectively in Group 1 and Group 2 and incubated for 1, 3, 24 and 48 hr. Optical densities of the suspensions were measured spectrophotometrically at 640 nm. The experiments were repeated three times and data obtained was statistically analyzed with one-way ANOVA and Kruskal-Wallis tests. Surface of a sample from each group was examined by scanning electron microscopy. The results for Vitremer showed that at 24 and 48 hr, Oral-B ($\bar{x}$=0.35, $\bar{x}$=0.31) and Chlorhexamed Fluid ($\bar{x}$=0.11, $\bar{x}$=0.17) and at 48 hr, Colgate ($\bar{x}$=0.15) caused a statistically significant decrease in bacterial adhesion when compared to their positive controls ($\bar{x}$=0.83, $\bar{x}$=1.4) ($\bar{x}$=0.8, $\bar{x}$=1.5) ($\bar{x}$=0.7). When Dyract was evaluated, significantly less bacterial growth was observed with Chlorhexamed Fluid at 24 hr ($\bar{x}$=0.17) compared to the group not treated with the mouthwash ($\bar{x}$=0.6) and at 48 hr, Oral-B ($\bar{x}$=0.39) and Colgate ($\bar{x}$=0.15) treatment showed a statistically significant decrease compared to the positive control group ($\bar{x}$=0.96, $\bar{x}$=0.71). For Dyract and Vitremer, Chlorhexamed Fluid treatment resulted in a significant decrease of the bacterial population when compared to Oral-B treatment at 24 hr. Hence, we conclude that Chlorhexidinedigluconat was the most effective antimicrobial agent among the ones investigated in this study.

## 2840 In Vivo Antibacterial Activity of High Fluoride Toothpaste on Dental Plaque Bacteria.

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Dental plaque is the primary aetiological agent for the development of dental caries and periodontal diseases. The purpose of this study was to determine the in vitro antibacterial activity of a high fluoride toothpaste (2500 ppm) as a mixture of sodium fluoride and sodium monofluorophosphate versus a placebo toothpaste. We evaluated the spectrum of antimicrobial activity from both formulation on 14 strains: 4 cariogenic bacteria (Actinomyces odontolyticus, Streptococcus mutans, Streptococcus salivarius, Lactobacillus casei) and from 10 periodontal microflora (Actinobacillus actinomycetemcomitans, Eikenella corrodens, Capnocytophaga ochracea, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Peptostreptococcus micros, Campylobacter rectus, Prevotella melaninogenica, Bacternides loeschii ...). The inoculum was a standard concentration of bacteria cells in a appropriate broth media. The minimum inhibitory concentration (MIC) of each toothpaste was evaluated on three dilutions: 1/2, 1/4, 1/8 in a suitable liquid media. Each dilution was exposed to the inoculum at three times for three different periods: 5 mn, 1 hour, 24 hours. The results were expressed as the mean of the three assays. The excipient presented a very weak inhibitory activity on the 14 strains. Sometimes, we observed bacteria growth with 24 hours time exposure and for the highest level of dilution. The facultatively anaerobic bacteria (Streptococcus sp., Lactobacillus sp. and Actinomyces sp.) were the most sensitive to the high fluoridated toothpaste with a reduction of growth by a factor from 2 to 110. For example, A. odontolyticus exposed to the high fluoride toothpaste during 24 hours decreased from $3 \times 10^6$ to $2 \times 10^4$. The capnophilic bacteria (Actinobacillis sp., Eikenella sp. and Capnocytophaga sp.) presented a medium sensitivity to the fluoridated toothpaste with a reduction factor of 1,5 to 17,5. The strictely anaerobic species presented the lowest sensitivity to the fluoridated toothpaste with a factor reduction between 1 and 11,2.

In conclusion, the high fluorided toothpaste tested in vitro demonstrated a significant inhibitory effect on the growth of the facultatively anaerobic bacteria on the dental plaque. Strict anaerobic and cannophilic bacteria are less sensible to the formula.

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